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MICROORGANISMS IN U V LIGHT

E.Petras & A.Ullrich

Microorganisms characteristically possess a high content of nucleic acids. In bacteria, nucleic acids may amount to 25% or more of their dry weight. Consequently, they absorb ultraviolet light to a high degree. This fact makes bacteria an interesting object in theoretical and applied UV irradiation biology, because they can be easily manipulated and because they multiply very rapidly.

In Fig.1 there are shown UV spectra of typical nucleic acid and protein preparations and of E. coli cells* Note that the absorption spectrum of the bacterial cells is almost identical with that of pure nucleic acid. Additional UV absorption is caused by the presence of cellular proteins. This explains also why the same bacterium in different growth phases produces different UV spectra: the percentage contents of DNA, RNA and protein vary considerably.

When exposed to UV light, the sensitivity of microorganisms is generally proportional to the intensity of the absorption at different wavelengths. It is shown in Fig.2 that the maximum effect of UV irradiation is at about 265 mμ.

Irradiation with UV light leads to mutations. In almost all cases, this kind of irradiation kills or inactivates cells. Inactivated cells are disturbed in their reproductive mechanism, cannot multiply and, sooner or later, perish. When visible light of proper wavelength is used, "reactivation" occurs and the cells resume normal multiplication(see Fig.3).

* A UV-microspectrograph, E.Leitz, Wetzlar, and a double-beam microdensitometer, Joyce, Loebl & Co., England, were used in these studies.

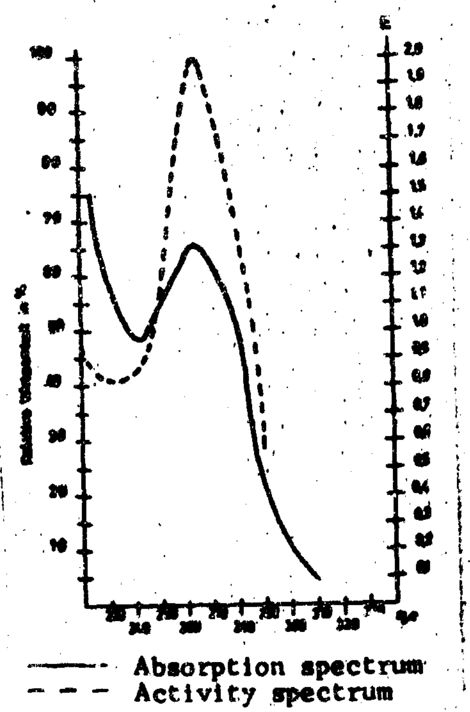


Fig.1. UV spectra of *E. coli*, nucleic acid and protein

Fig.2. Sensitivity spectrum and UV absorption spectrum of *E. coli*

Through "photoreactivation" the rate of survival of UV irradiated bacteria can be increased, sometimes, more than 10,000-fold. Certain microorganisms can also be reactivated by heat.

The biological activity of UV irradiation appears to produce an interlacing effect (Vernetzung) within the DNA molecule, while reactivation appears to undo it.

As shown in Fig.2, the sensitivity of certain micro-organisms to UV rays can be determined by calculating the percentage of cells unable to grow on a certain medium, after irradiation, in relation to a given population, regardless of whether we are dealing with dead, inactivated and eventually mutated cells with special nutritional requirements. Actually,

the differentiation is difficult to determine. When a cell cannot be reactivated, it does not mean it is dead. A cell may possess normal, native nucleic acids - and nowadays this can be ascertained by methods of nucleic acid hybridization - but it may be more or less strongly altered in its protein structure. Such cells are not unequivocally "dead" whenever they are unable to grow on a suitable medium. There are no exact methods which will differentiate between live and dead microorganisms because of lack of precise criteria. However, the interior of a microbial cell can be studied to a considerable extent by present methods, especially when several methods are combined. Upon heat-fixation, the UV absorption spectrum changes, not necessarily quantitatively, but empty and autolyzing cells can be readily spotted by their lack of UV absorption.

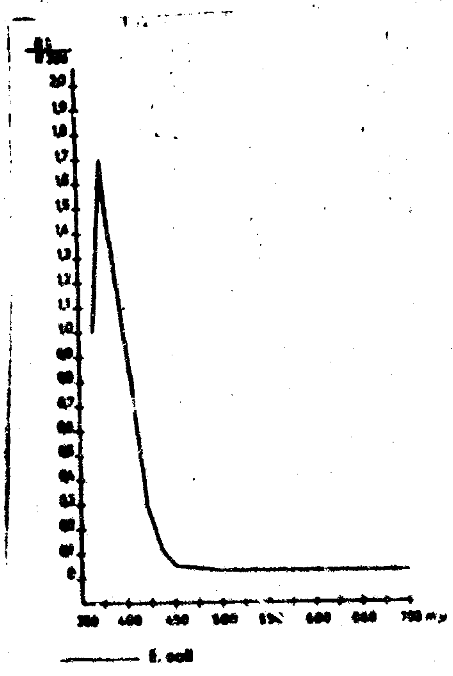
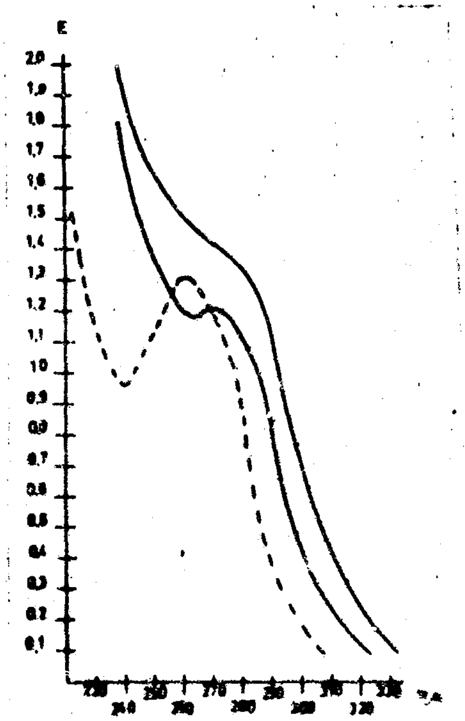


Fig.3. Activity spectrum of the photoreactivation of E. coli



—— E. coli in skim milk
- - - E. coli alone (control)

Fig.4. UV spectra of E.coli cells coated with two concentrations of skim milk. Normal UV spectrum for comparison.

Whether and how microorganisms are able to protect themselves against UV rays is an interesting question. The cell must be able to do it, because it is known that in high altitude atmospheric layers, where UV rays of great intensity and constantly and naturally present, there exist large numbers of viable bacterial and fungal cells. Undoubtedly, photo-reactivating processes must play a role. Presumably, there exists a mechanism which in visible light activates an enzyme which eliminates the interlacing process (Vernetzung) within the DNA molecule. Pigments are common in air organisms and may play a role in providing the reactivating enzyme with a suitable light spectrum or by providing some other mechanism. It is possible that other protective mechanisms exist, e.g. cell envelopes which absorb UV rays or distortions of cell structures which prevent the interlacing process in the DNA molecule.

The use of UV spectroscopy is important in the search for life in the atmosphere and on other planets. The basic assumption must be made that extraterrestrial life also depends on nucleic acid and protein complexes. If this is accepted as a working hypothesis - and there are no better criteria for explaining "life" - no other theoretical difficulties are then encountered. It is possible that spectra of nucleic acids are masked by minute amounts of other substances (see Fig.4), but this problem can be solved with presently available methods.

Within the near future, perhaps, in this manner, new insights will be gained about life.